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Genomic imprinting proposed as a surveillance mechanism for chromosome loss

(cancer/monosomy/trisomy)

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ABSTRACT **One consequence of genomic imprinting is that loss of the transcriptionally active chromosomal homologue causes a change in gene expression that might permit surveillance of chromosome-loss events. Possible selective advantages of such surveillance include protection against cancer and early elimination of monosomic and trisomic fetuses. Potential mechanisms for such surveillance are discussed.**

Genomic imprinting as used in this paper means the condition in which the normal alleles of a gene display distinct expression patterns. Typically, the allele inherited from one parent is transcribed while the allele from the other parent is not. This state is stably maintained during the cell divisions of development, with the result that most or all cells in an individual display the same allele-restricted expression. The imprinting state of alleles can be reversed during gametogenesis, demonstrating that the state is epigenetic. This imprinting phenomenon has been clearly demonstrated for several genes in mice and humans and probably exists widely in mammals (1–7). Differential methylation of DNA is correlated with imprinting for all genes thus far analyzed and probably causes the differential transcription of alleles (8). It appears that the hypermethylated allele of imprinted genes can be either the expressed or the unexpressed allele (8, 9). I will refer to a locus that is subject to imprinting as an imprinted gene, and to the unexpressed allele as the imprinted allele, regardless of its methylation state. For a particular gene, the imprinted allele is always inherited from the parent of one sex, but both paternal and maternal allele imprinting have been described and thus far the two appear to be about equally common. A recent genomic survey for genes imprinted by differential methylation (10) suggests that there are about 100 imprinted loci in the mouse. Most of this paper will concern the mouse, since imprinting is best studied there, but many of these thoughts may apply widely to mammals.

Imprinting is a phenomenon in search of a reason. Although some interesting hypotheses have been advanced to explain the evolution of imprinting (e.g., refs. 11–13), no consensus about the correct explanation has yet been reached. The purpose of this paper is to propose an explanation for the function of imprinting and to explore its consequences and predictions.

Proposal of a Surveillance Mechanism for Chromosome Loss. If imprinted alleles are present randomly on both paternal and maternal chromosomes and they are dispersed fairly evenly throughout the genome, one consequence of imprinting about 100 loci in the mouse is that most or all chromosome-loss events will result in complete loss of expression of at least one gene. This fact raises the possibility that imprinting provides a mechanism for detecting such chromosome-loss events. Under normal diploid conditions without imprinting, detection of chromosome-loss events may be problematic, since both the DNA amount and the expression of affected genes are reduced by only a factor of 2. However, if each homologue of a chromosome is the sole source of expression of one or more genes, then loss of a homologue will result in elimination of expression of those genes. Such a loss of expression could be actively detected by the cell involved, resulting in an appropriate cellular response, or the loss of expression could directly affect cell function. Chromosome-loss events are known to have deleterious consequences that imprinting could serve to prevent. The next sections discuss two such deleterious consequences that might be avoided by such a surveillance mechanism.

Protection from Cancer. Currently favored theories for the ontogeny of cancer propose that a primary step toward the development of cancer is the acquisition of genomic instability (e.g., ref. 14). This genomic instability favors the accumulation of the many genetic changes required to generate malignancy (15). This consequence of genomic instability should provide strong selection for high-fidelity somatic replication and repair of DNA and chromosome segregation, especially for the prevention of childhood cancers, which strongly affect reproductive fitness. While such fidelity mechanisms undoubtedly reduce cancer risk from genomic instability, it is also clear that they often fail. Another way in which the consequences of genomic instability might be ameliorated is to detect changes after they occur and to orchestrate appropriate action to prevent their deleterious consequences. Genomic imprinting could provide a simple mechanism for surveillance of large deletions, whole chromosome losses, or other events that result in loss of heterozygosity. Such a mechanism would be most effective in detecting large-scale loss of heterozygosity, since such events would tend to affect more imprinted genes. Though it is unlikely to be based on an imprinting mechanism, an interesting precedent for chromosome segregation surveillance has been demonstrated: when a chromosome rearrangement causes abnormal mitosis in the early *Drosophila melanogaster* embryo, the resulting nuclei are selectively eliminated from the dividing population of nuclei that will form the embryo (16).

Imprinting might reduce cancer risk by various mechanisms. One interesting hypothetical mechanism would require the existence of a regulatory system of gene-expression detectors whose function is to block cell division or activate cell death in response to loss of expression of an imprinted gene. For example, each cell might be under continual surveillance by policing cells of the immune system, which would demand presentation of a set of cell surface products as a license to live. In this model, imprinted genes would encode such cell surface products or would regulate their synthesis or secretion. A precedent for such an active mechanism has been described for response to DNA damage. Cell death in response to ionizing radiation and other DNA-damaging agents results not directly from cellular damage, but from activation of programmed cell death (e.g., refs. 17 and 18).

An alternative mechanism to reduce cancer risk could involve direct consequences of the loss of expression of an

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imprinted gene. For example, each chromosome might carry one paternally and one maternally imprinted gene whose expression is required for cell division. If this were the case, then genomic instability, detected by loss of any paternal or maternal chromosome, would result directly in growth arrest of the affected cell. In addition to genes required for cell division, this mechanism could act through genes for essential cellular housekeeping functions, cell-death suppressor genes, and genes that favor metastasis. Imprinting could also act on genes whose expression favors the probability of cancer, rather than those absolutely required for cell proliferation. Such genes need not favor cancer in all tissues, though natural selection would favor imprinting of widely important genes.

Fetal Chromosome Imbalances. A second condition in which chromosome loss is especially deleterious is when it occurs during meiosis, resulting in a fetus that is monosomic or trisomic. Such an event is nearly always lethal to the fetus in mammals, and early detection and abortion of monosomic and trisomic fetuses might be advantageous. Of all animals, females of placental mammals have a uniquely high cost associated with reproduction. They provide an unusually large amount of nutritional support prior to birth and are potentially at health risk from fetal abnormalities that cause spontaneous abortion, especially late in gestation. Approximately 30% of all human conceptions end in spontaneous abortion (19), and approximately half of these fetuses have gross chromosome abnormalities, among which trisomies and monosomies dominate (e.g., refs. 20 and 21), though other mammals may have much lower rates of fetal chromosome abnormalities (e.g., ref. 22). Early detection and abortion of monosomic and trisomic fetuses could provide a selective advantage by avoiding nutritional support for the doomed fetus, by reducing health risk to the mother, or by expediting a new normal pregnancy.

Genomic imprinting could provide an effective mechanism for facilitating early abortion. In a chromosomally normal fetus, imprinting results in expression of only the maternal or paternal allele of a particular gene. Fetuses that are monosomic and carry only an imprinted allele on the remaining chromosome will be functionally deficient for the imprinted gene. If loss of function of the imprinted gene causes fetal abnormalities, a direct consequence may be earlier spontaneous abortion of the monosomic fetus. The loss of expression of an imprinted gene need not itself result in fetal lethality in order for the imprint to be a selectable trait. Monosomy itself has direct deleterious effects caused by abnormal gene dosage on the affected chromosome. Selection will favor a particular imprint as long as failure to express the imprinted gene results in *earlier* (lower-cost) abortion than that caused by the monosomy alone.

Trisomy detection could also be facilitated by imprinting. The consequence of inheriting two copies of a nonimprinted allele will be the expression of the imprinted gene at 2 times the normal expression level, instead of the 1.5 times that would result from simple nonimprinted trisomy. This increased dosage might be enough to enhance lethality of the already deleterious triple dose of the other trisomic genes. A more effective mechanism for trisomy detection would involve activity titration by equally expressed but reciprocally imprinted linked genes. For example, if two such gene products form a heterodimer, trisomy arising from duplication of either parental homologue would result in the liberation of one of the monomer species. The liberated monomer could be detected and an appropriate response generated. It is interesting that the imprinted gene *H19* has been shown to cause embryo lethality when expressed in just two copies (ref. 28 and S. Tilghman, personal communication).

Experimental Tests. The ideas proposed here are speculative and demand experimental testing. I will conclude by mentioning the few pertinent existing experimental results and by proposing some potentially rigorous tests. A partial test of the cancer-protection hypothesis is available, using uniparental chromosome inheritance in the mouse. Ideally, large numbers of animals with uniparentally derived chromosomes would be assessed for quantitative effects on cancer incidence. In limited tests of chromosomal segments totaling about half the mouse genome, no obvious predilection to cancer was reported (1, 23–25). This negative result is inconclusive for several reasons. (*i*) Because of the translocation method used, it was not possible to test the consequences of uniparental inheritance of any whole chromosomes. (*ii*) Because of lack of appropriate translocations and the fact that uniparental inheritance of some chromosomal segments caused embryonic lethality, it was not possible to test the entire genome. (*iii*) Small numbers of animals carrying uniparentally derived segments were studied and no report was made of attempts to assess cancer rate. Given the probable presence of several dispersed imprinted genes on each chromosome and the likelihood that redundant systems function to reduce cancer risk, it is not clear how much cancer rates would be elevated in the absence of the hypothesized chromosome-loss surveillance. Another test of the cancer-protection hypothesis is to study the methylation-defective mouse mutant which has recently been identified and may lack imprinting altogether (8). The methylation-defective mouse is homozygous inviable, but cultured mutant embryonic stem cells are viable. Cancer rates in methylation-deficient cells might be assessed either by introducing mutant embryonic stem cells into otherwise normal mice or by the identification of less severe methylation-deficient alleles with improved viability.

Some recent reports are consistent with imprinting playing a cancer-protective role in humans. Rainier *et al.* (6) and Ogawa *et al.* (7) have shown that inappropriate expression of both alleles of the imprinted genes *H19* and *IGF2* is commonly found in Wilms tumors. These authors hypothesize that changes in functional gene dosage play a causative role in Wilms tumor formation, but the chromosome-loss surveillance hypothesis can also account for their findings. Specifically, loss of imprinting of *H19* and *IGF2* prior to tumor formation could cause a failure in chromosome-loss surveillance. This failure in surveillance, rather than any specific change in gene dosage, might predispose to tumor formation. Uniparental disomy in the cancer-predisposing Beckwith–Weideman syndrome (26) and preferential mutation of one parental allele in retinoblastoma (27) have also been described. If the cancer-protection hypothesis is correct, there should exist a correlation in some tumors between the extent of loss of heterozygosity and the positions of imprinted genes.

Tests of the fetal chromosome-imbalance hypothesis may be currently more difficult to perform. As described above, a viable imprinting-defective mouse mutant would be ideal for such tests, but this may not be technically feasible. Alternatively, it should be possible in principle to use transgenic technology to engineer a single chromosome in the mouse on which all imprinted loci have lost their imprinting. Studies of fetuses that are monosomic and trisomic for this chromosome in both the wild-type and the engineered mouse would conclusively address the fetal chromosome-imbalance hypothesis.

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